REMARKS

Upon entry of this Amendment, claims 1-20 will be pending in the present application. Claims 5-8, 17 and 18 were previously withdrawn from consideration. Claim 1 is herein amended. No new matter has been entered. It is respectfully submitted that this Amendment is fully responsive to the Office Action dated April 29, 2008.

Claim Rejections - 35 U.S.C. §103(a)

Claims 1-4, 9-16 and 19-20 were rejected under 35 U.S.C. §103(a) as being unpatentable over the combination of *Tsai et al.* in view of *Tanaka et al.* (Wat. Res. 1997; 31 (8): 1913-1918). Applicants respectfully disagree with the examiner's characterizations of the cited references and request that the rejection of claims 1-4, 9-16 and 19-20 be withdrawn.

In rejecting claim 1, the examiner acknowledged that *Tsai* does not teach a second syringe, the particular washing liquid as claimed, or the using of sterile distilled water to make the solid sample into liquid sample. However, the examiner asserted that *Tanaka* teaches a measuring kit of microorganisms that filters with a suction pump. Accordingly, the examiner concluded that it would have been obvious to modify the *Tsai* method by using a second syringe, using ethanol as taught by *Tanaka* because the two kits are each used to measure microorganisms.

Applicants disagree with the examiner's reasons for rejecting claim 1. In particular, Applicants submit that it would not have been obvious to modify *Tsai* to include an additional

suction pump, as purported above by the examiner. In order to satisfy this missing element of the claimed invention, the Examiner appears to find it implied by the disclosure in *Tsai* that:

> "bacteria passing through the upstream filter were concentrated on the small pore size filter (0.4-μm pore, 13-mm diameter). Soluble ATP and other interfering substances which escaped both filters were voided as the end filtrate. The filters were washed with the phosphate buffer prior to their retrieval for bioluminescence assay."

(Tsai page 75, last sentence to page 76, first paragraph)

However, the **second syringe** stores washing liquid (*i.e.* sterile distilled water with 60-100% ethanol or 1-8% DMSO) used to wash the second filter case. Whereas, *Tsai* teaches that the filters were washed but does not teach that the phosphate buffer used to wash a second filter came from a syringe. Furthermore, since the phosphate buffer washing liquid disclosed in *Tsai* is distinct than the washing liquid of sterile distilled water with 60-100% ethanol or 1-8% dimethylsulfoxide (DMSO) disclosed in Applicant's specification there can be no inference that phosphate buffer would require the use of a syringe to inject it upon the second filter simply because that method may be useful for the application of another very different type of washing solution.

Moreover, Applicant's specification teaches that the second syringe deposits the bacteriolytic agent ([0030]). Whereas, *Tsai* discloses that "an aliquot of 150μl of lysostaphin (10 units/ml) was added to the filter on which bacterial cells were collected." Lysostaphin is the biological bacteriolygic agent used in *Tsai*. *Tsai* does not disclose that a second syringe is used to obtain the aliquot. If a syringe is implied in these excerpts, it would seem the first and only syringe disclosed is used.

Although *Tsai* teaches a washing liquid, the phosphate buffer disclosed in *Tsai* is not the sterile distilled water with 60-100% ethanol or 1-8% dimethylsulfoxide (DSMO) taught by Applicant. Whereas, for example, Applicant's specification discloses that using phosphorus compounds such as phosphoric acid as a flocculant "coats the microorganisms" such that "at the time of extracting the ATP, the bacteriolytic agent is hard to work. Then, it is indispensable to wash with a large quantity of washing liquid. As a result, there is a fear that it becomes a factor of instability of a test result." (See Specification [0010] and [0130].)

Furthermore, with regards to the "second syringe," *Tanaka* discloses "The suspension was filtered through a filter with a suction pump and washed 2-3 times with the cleaning solution." See the last paragraph of the left column to the first paragraph of the right column of page 1914 and Fig. 2 of *Tanaka*. However, this description does not describe that the suction pump is also used for cleaning of the filter after filtering bacteria. Rather, as disclosed on page 1914 and in Fig. 2 of *Tanaka*, the suction pump is used in the filtration step for filtering the bacteria suspension through the filter (e.g., "filtration under pressure" or the second step in Fig, 2). The washing step, for example, (third step in Fig. 2) is recited as the step subsequent to the filtration step. In other words, the filter is "washed 2-3 times with the cleaning solution" after the bacteria suspension was filtered through the suction pump. *Tanaka* does not disclose or even suggest that the suction pump is used for the washing step. Moreover, referring to the diagram shown in Fig. 2 of Tanaka, the washing step is clearly differentiated from the filtration step as the step (third step) next to the filtration step (second step).

In view of the aforementioned remarks, Applicants submit that neither of the cited references discloses a second filter as described in the claimed invention (*e.g., Tanaka* never teaches or suggests the use of the syringe in the washing after filtration as in the present invention, particularly the second syringe which attaches the second filter case thereon and used for washing the second filter case with the washing liquid.)

Furthermore, Applicants submit that neither of the cited references discloses a measuring kit having "a flocculant for flocculating protein in the liquid sample in the first syringe." See claim 1. The flocculant flocculates only the protein in the liquid sample in the first cylinder but never flocculates the microorganisms. Thus, when the first filter case is attached to the first syringe, the first filter housed in the first filter case traps or catches only the flocculated protein, while passing or transporting the microorganisms therethrough. Then, when the second filter case is attached to the first filter case, the second filter housed in the second filter case traps only the microorganisms, since all the protein is caught in a flocculated manner on the first filter at an upstream side. Specifically, Applicants submit that "free ATP, FA, somatic cells BC (containing ATP therein), protein PR and the like are mixed in a liquid sample LS such as a milk or the like in addition to microorganisms MO." Therefore, "unless these substances are removed from the liquid sample LS, it is impossible to measure a mixed amount of the microorganisms MO with accuracy." *See* paragraphs 0070 of the specification of the present application. Among these interfering substances, since the somatic cells have larger size than the microorganisms, they can be filtered and separated from the microorganisms by using a first filter having pore size larger than the microorganism and smaller than the somatic cell. On the other hand, since the free ATP

is soluble in the water, it can be separated from the microorganisms by using a second filter having pore size smaller than the microorganism. Still, since the protein has smaller size than the somatic cell and is not soluble in the water, it possibly passes the first filter together with the microorganisms and is filtered or caught on the second filter together with the microorganisms. Therefore, it is necessary to catch the protein on the first filter, while allowing the microorganisms to pass through the first filter.

Tsai does not disclose, teach or even suggest that protein interferes or deteriorates a measurement of the microorganisms and that the protein should be filtered or removed before the microorganisms are caught on a filter. Likewise, *Thai* does not disclose or even suggest a measuring kit comprising a device or means for filtering or removing the protein before catching or filtering the micro organisms. Whereas, in the present invention, the flocculant for flocculating the protein in combination with the first filter is used as such device or means for filtering or removing the protein before catching or filtering the microorganisms. Accordingly, the present invention is able to separate the protein from the microorganisms without fail and to collect only the microorganisms.

Furthermore, the Examiner mischaracterized what is disclosed in paragraph 0007 of the specification of the present application (e.g., alleging that the "phosphoric acid buffer agent" of *Thai* is broadly interpreted as a flocculant). Applicants submit that the "phosphoric acid buffer agent" (recited as "phosphate buffer" in *Tsai*) disclosed in *Tsai* is merely a buffer used for making "Serial dilutions from an aliquot of the stock broth" (page 74, right column, last two lines) or used for cleaning the filters (page 76, right column, lines 4-6). Also, the corresponding

part (page 14, lines 5-6) of Applicant's response filed 1/22/2008 only describes that the "phosphoric acid buffer agent" is used "for making the solid sample into, the liquid sample." In other words, this means that the "phosphoric acid buffer agent" is a buffer used for making "Serial dilutions from an aliquot of the stock broth." Whereas, the "phosphate buffer" of *Tsai* has a completely different function and results from the function (flocculating action) and results of common flocculants. The "phosphate buffer" of *Tsai* is not a substitution or equivalent substance to the flocculant. Although paragraphs 0007 and 0008 of the specification show a phosphoric acid flocculant, *Tsai* has no consideration on the "protein" or its treatment or on "flocculation" of any substance. Thus, *Tsai* does not disclose, teach, or suggest the "flocculation" of "protein." Accordingly, a person skilled in the art is would not be motivated to use the "phosphate buffer" of *Tsai* as a flocculant, particularly as a flocculant for protein.

Assuming *arguendo* that the "phosphate buffer" of *Thai* is a flocculant, the phosphoric acid flocculant is not suitable in case of measurement of the microorganisms, since it flocculates not only the protein but also the microorganisms. For example, in such situation, "an unskilled person may flocculate protein and microorganisms without distinction at the time of flocculating reaction so as to lose the microorganisms in the sample", as described in the paragraph 0008 of the specification. That is, part of the microorganisms are flocculated and trapped or caught on the first filter, thereby decreasing an amount of the microorganisms that are caught on the second filter for measurement. Moreover, there is caused another problem that "since the phosphoric acid flocculant coats the microorganisms at the time of extracting the ATP, the bácteriolytic agent is hard to work. Then, it is indispensable to wash with a large quantity of washing liquid.

As a result, there is a fear that it becomes a factor of instability of a test result." See, for example, paragraph 0008 of the specification.

As discussed in the specification, one object of the present invention is to overcome the drawbacks of using the phosphoric acid flocculant in measuring microorganisms in a liquid sample. In order to solve these problems, the present invention uses the "flocculant for flocculating protein in the liquid sample in the first syringe", *i.e.* the flocculant for flocculating the protein but microorganisms.

In view of the aforementioned remarks, Applicants submit that neither of the cited references discloses *a flocculant for flocculating protein in the liquid sample in the first syringe*. Accodingly, in view of the aforementioned remarks, Applicants request that the examiner withdraw the obviousness rejection of claim 1.

Applicants acknowledge that claim 1 does not explicitly recite the words "only (protein)." However, Applicants submit that it is clear from the claim language (e.g., claim 9) and the specification (paragraphs 0008, 0009, 0010 and 0026-0029) that the "flocculant for flocculating protein in the liquid sample in the first syringe" is the flocculant for flocculating **only the protein**. Nevertheless, to expedite prosecution, Applicants herein amend claim 1 to clarify that the measuring kit comprises *a flocculant for flocculating only protein in the liquid sample in the first syringe*. Support is found, for example, in the paragraphs identified above. Applicants submit that this clarifying amendment should not necessitate a new search. Accordingly, in view of this amendment and the aforementioned remarks, Applicants request that the obviousness rejection of claim 1 be withdrawn.

Furthermore, Applicants submit that claim 9 is allowable over the combination of references. None of the cited references discloses the specific flocculants for flocculating protein that are recited in the claim. Accordingly, Applicants request that the rejection of claim 9 be withdrawn.

Claims 2-4, 9-16 and 19-20 depend from independent claim 1. Applicants request that the rejection of these claims be withdrawn in view of the aforementioned remarks distinguishing claim 1 from the cited references.

Conclusion

In view of the aforementioned amendments and accompanying remarks, Applicants submit that the claims, as herein amended, are in condition for allowance. Applicants request such action at an early date.

If the Examiner believes that this application is not now in condition for allowance, the Examiner is requested to contact Applicants' undersigned attorney to arrange for an interview to expedite the disposition of this case.

If this paper is not timely filed, Applicants respectfully petition for an appropriate extension of time. The fees for such an extension or any other fees that may be due with respect to this paper may be charged to Deposit Account No. 50-2866.

Respectfully submitted,

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